Award Number: W81XWH-13-1-0091

TITLE: Targeting Androgen Receptor in Breast Cancer: Enzalutamide as a Novel Breast Cancer Therapeutic

PRINCIPAL INVESTIGATOR: Dr. Anthony Elias

CONTRACTING ORGANIZATION: The Regents of the University of Colorado
Aurora, CO 80045

REPORT DATE: September 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
September 2014

2. REPORT TYPE
Annual

3. DATES COVERED
15Aug2013 - 14Aug2014

4. TITLE AND SUBTITLE

Targeting Androgen Receptor in Breast Cancer: Enzalutamide as a Novel Breast Cancer Therapeutic

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0091

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University of Colorado

Aurora, CO 80045-2505

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Enzalutamide has clinical activity in breast cancer as a single agent and in combination with exemestane. Activity is seen in both triple negative AR+ BC and also ER+AR+ BC. Clinical data in Her2+ AR+ BC is too immature to make conclusions. The proposed clinical trials for Years 3-5 appear to be justified based on clinical activity and the current preclinical data.

15. SUBJECT TERMS
Breast cancer (BC) subtypes; androgen receptor (AR); preclinical modeling; enzalutamide; AR inhibition; resistance mechanisms; predictive biomarkers.

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
Unclassified	Unclassified	Unclassified	Unclassified	13	USAMRMC
					19b. TELEPHONE NUMBER *(include area code)*

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Award Number W81XWH-13-1-0091 Annual Report (Year 1)

Collaborating/Partnering PI

Anthony D Elias, MD

Introduction

The central thesis of this grant is to understand the role of AR signaling in breast cancer subtypes, and understand how to best use an inhibitor of AR signaling, enzalutamide (enza), as a therapeutic agent in breast cancer. With the recognition that AR is expressed in all subtypes of breast cancer, that overexpression is frequently associated with relative resistance to therapy (both anti-estrogen and chemotherapy) (work of our group and others), and with the advent of increasingly potent AR signaling inhibitors in prostate cancer, the area of anti-AR therapeutics in breast cancer is one of the most active worldwide. The preclinical portion of this grant serves to understand mechanism of action of AR signaling inhibition alone or in combination with other targeted agents in ER+, Her2+, or TNBC in preclinical models, and then perform biomarker analysis in human tissues obtained before, during and after treatment with enzalutamide. The clinical portion of this grant serves to obtain these tissues in concert with the overall clinical development of enzalutamide in the subtypes of breast cancer.

Keywords

Breast cancer (BC) subtypes; androgen receptor (AR); preclinical modeling; enzalutamide; AR inhibition; resistance mechanisms; predictive biomarkers.

Overall Project Summary

This report will include both the tasks that were mandated in the SOW, but also will highlight the therapeutic clinical results from the companion therapeutic trials that were sponsored by our Pharma partners, Medivation and Astellas.

Clinical Aim 1: To identify pretreatment molecular characteristics associated with lack of response and/or prolonged PFS (Patient Tissues).
Task 1: Write & Activate the Serial Biopsy Trial (Elias, LoRusso, Traina, advocates, Richer)

- Written protocol completed Month 1
- Submitted to Scientific Review Committee Month 1
- Submitted to IRBs (all institutions) Month 1
- DoD Human Research Protection Office (HRPO) Month 2
- Activation and first patient enrolled Month 3
- **IND already available**

This task was completed on time. The DOD sponsored serial biopsy trial has been written and approved by HRPO and has been activated at University of Colorado site. Its title is “Exploratory Development of Predictive Biomarkers for Patients with Androgen-Receptor Positive (AR) Breast Cancer (BC) Treated with Enzalutamide (MDV3100); COMIRB 13-1473. The same trial is about to be IRB approved at MSKCC (Traina, local PI). MSKCC has been accruing well to the therapeutic trials and have all the new trials activated. Thus that site will continue to participate. Due to changes in personnel (Dr. LoRusso moved to Yale Cancer Center), Karmanos Cancer Center was deactivated, and with the approval of the Dept of Defense, the University of Tennessee is in the process of being activated (PI, Lee Schwartzberg, MD). They have also been top accruers to the enzalutamide breast cancer trials.

Task 2: Accrue 12 patients treated with enzalutamide onto serial biopsy trial (Elias, LoRusso, Traina, advocates) Year 0-Year 2 Month 7

- First 12 patients accrued completed (12) Month 7
- First 12 patients – clinical database complete Month 15
- All initial biopsies collected from 1st 12 patients Month 12

The first patient was accrued in 10/2013. Thus far, five patients have been accrued to the serial biopsy trial (all at University of Colorado). There were two screen failures. We do expect that the accrual will accelerate once MSKCC and University of Tennessee have opened this trial. All five patients have adequate tissue obtained from pretreatment and 2-4 weeks into treatment. One patient has provided post-progression tissue and two have refused. Two patients remain on therapy.

Task 3: Tissue assays and bioinformatics analysis (Richer, Thor, Jones, Elias, LoRusso, Traina, Petricoin, Gao)

- First 12 patients completed Month 18
- Bioinformatic analysis Month 24

The timeline for this task has not yet been reached.

Clinical Aim 2: To determine if a decrease in Ki67 or increase in apoptosis as measured by TUNEL in biopsies taken before treatment as compared to after 2-4 weeks of treatment or other to be determined genes or proteins are associated with lack of response and/or prolonged PFS.

Task 1: Accrue 24 patients treated with single agent enzalutamide (Elias, LoRusso, Traina, advocates)

- First half of patient accrual completed (12) Month 15
- First 12 patients – clinical database complete Month 15
- All 2-week biopsies collected from 1st 12 patients Month 15

Task 2: Tissue assays and bioinformatics analysis (Richer, Thor, Jones, Elias, LoRusso, Traina, Petricoin, Gao)

- 12 single agent patients Month 18
- Bioinformatic analysis completed Month 24

The timeline for these tasks have not yet been reached.

Clinical Aim 3: To determine if changes in molecular determinants between pre-treatment biopsies and tissue at time of disease progression can help identify resistance mechanisms.

Task 1: Accrue 24 patients treated with single agent enzalutamide (Elias, LoRusso, Traina, advocates)

- All relapse biopsies collected from 1st 12 patients Month 24

The timeline for this task has not yet been reached.

Clinical Aim 4: To determine if enza can overcome *de novo* resistance to exemestane in postmenopausal women with T2 or larger ER+ BC treated preoperatively.

Task 1: Trial II: Randomized Preoperative trial in AR+/ER+ BC (Elias, LoRusso, Traina, advocates, Richer)

- Written protocol completed Month 21
- Submitted to Scientific Review Committee Month 21
- Submitted to IRBs (all institutions) Month 22
- DoD Human Research Protection Office (HRPO) Month 24

The clinical development of enzalutamide in breast cancer has been rapid. Both Medivation and Astellas have been fully committed to this endeavor. Findings are summarized:

- The phase I of single agent enzalutamide has been completed and confirmed that the FDA approved dose in prostate cancer in men (160 mg daily) is safe and tolerable in women. Additionally, the pharmacokinetic profile of enzalutamide in women is similar to that in men.
- Because enzalutamide is a very strong p450 CYP3A4 inducer, several phase Ib trials have been completed to examine the pharmacologic interaction of enza with other anti-estrogen agent (anastrozole, exemestane) in ER+ BC.
- Enzalutamide when added to anastrozole 1 mg daily reduced the AUC of anastrozole alone by 80%. This was associated with an increase in serum estradiol in some patients. For this reason, this combination is no longer in development.
- Enzalutamide when added to exemestane 25 mg daily reduced the AUC of exemestane by about 50%. This was not associated with an increase in estradiol. However, since the FDA approval for exemestane included approval for double dose exemestane (50 mg daily) when combined with strong CYP3A4 inducers, enza plus exemestane 50 mg daily was evaluated. Pharmacokinetic analysis of this combination demonstrated that exemestane 50 mg AUC (when combined with enza) was equivalent to exemestane 25 mg daily alone. As presented at ASCO 2014. Of 39 evaluated patients, 12 remain on therapy for more than 16 weeks (range 114-450 days). Thus this combination is moving forward in development.
- A current PK trial combining enza with fulvestrant is nearing completion.
- The initial immunohistochemistry assay for AR used a 10% staining cutoff to determine positivity. With the observation preclinically that cell lines that had lower levels of AR expression were sensitive to enzalutamide inhibition, the more recent clinical trials are now using a cutoff of 1% to select eligible patients.
- A randomized double-blinded phase II trial of exemestane with or without enzalutamide in women with metastatic ER+ AR+ breast cancer is underway and is open at each of the sites involved with this grant. Upon progression, an open label combination therapy is available for patients who had single agent exemestane initially. These latter patients are eligible for our ongoing DOD grant biopsy trial.
- A phase Ib of single agent enza in AR+ TNBC was completed. Currently a phase II trial is underway. These patients are eligible for our ongoing DOD grant biopsy trial.
- Based on our preclinical work, a trial of enzalutamide plus trastuzumab has opened in 3rd or greater line Her2+ AR+ BC. These patients are eligible for our ongoing DOD grant biopsy trial.

Clinical Aim 5: To determine the maximum tolerated dose and toxicity of enza when combined with the most promising combinations as defined in the preclinical modeling experiments during Years 1-2. As an example, a combination of enza with everolimus +/- a chemotherapy agent in previously treated metastatic TNBC.

Task 1: Trial III: Phase I/II trial in AR+/TN BC: Enzalutamide plus everolimus (Traina, Elias, LoRusso, advocates, Richer)

- Written protocol completed Month 21
- Submitted to Scientific Review Committee Month 21
- Submitted to IRBs (all institutions) Month 22
- DoD Human Research Protection Office (HRPO) Month 24

The timeline for this task has not yet been reached.

Milestone Meeting Month 21

Key Research Accomplishments:

Nothing to report. Tissue collection ongoing. Enzalutamide has clinical activity in breast cancer as a single agent and in combination with exemestane.

Conclusion:

Enzalutamide has clinical activity in breast cancer as a single agent and in combination with exemestane. Activity is seen in both triple negative AR+ BC and also ER+AR+ BC. Clinical data in Her2+ AR+ BC is too immature to make conclusions. The proposed clinical trials for Years 3-5 appear to be justified based on clinical activity and the current preclinical data.

Publications, Abstracts, and Presentations:

Papers:
Dawn R. Cochrane, Sebastian Bernales, Britta M. Jacobsen, Diana M. Cittelly, Erin N. Howe, Nicholas C. D'Amato, Nicole S. Spoelstra, Annie Jean, Paul Jedlicka, Kathleen C. Torkko, Andy Protter, Anthony D. Elias and J. K. Richer. Role of the Androgen Receptor in Breast Cancer and Preclinical Analysis of Enzalutamide. BREAST CANCER RESEARCH 2014 Jan 22;16(1). PMID: 24451109

Designated as Highly Cited by the journal Breast Cancer Research.
Submitted:
Barton VN, D'Amato NC, Gordon MA, Lind HT, Spoelstra NS, Babbs B, Heinz RE, Elias A, Jedlicka P, Jacobsen BM, Richer JK. Multiple molecular subtypes of triple negative breast cancer depend on androgen receptor for proliferation and invasion. **Submitted, September 2014**.

Abstracts:
D'Amato, NC, D Cochrane, N Spoelstra, A Chitrakar, B Babbs, A Protter, AD Elias, and J Richer. (Mar 2014) Inhibiting Androgen Receptor Nuclear Localization Decreases ER Activity and Tumor Growth in ER+ Breast Cancer. University of Colorado Postdoctoral Research Day, Aurora, CO. * won best overall poster award.

Barton VN, D'Amato N, Gordon M, Elias, A, and JK Richer. Targeting androgen receptor decreases proliferation and invasion in preclinical models of triple negative breast cancer. Presented at University of Colorado Cancer Center Annual Retreat "Novel Experimental Models for Cancer Research," September 2014. * Won outstanding poster award.
Elias A, Richer JK, LoRusso P, Peterson AC, Steinberg J, Mordenti J, Lopez C, Hudis C, Traina T. MDV3100-08: A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of MDV3100 in women with incurable breast cancer. ASCO 2012, TPS668.

Traina TA, Yardley, DA, Patel M, Schwartzberg L, Elias A, Gucalp A, Peterson AC, Hannah A, Gibbons J, Khondker Z, Hudis CA, LoRusso P. A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of enzalutamide (previously MDV3100) alone or in combination with an aromatase inhibitor in women with advanced breast cancer. SABCS 2013 PD3-6 (A938), accepted, poster discussion.

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Schwartzberg LS, Yardley DA, Elias A, Patel MR, Gucalp A, Burris HA, Peterson AC, Hannah AL, Blaney ME, Gibbons J, Tudor IC, Steinberg JL, LoRusso P, Infante JR, Hudis CA, Traina TA. Enzalutamide plus exemestane: a pilot study to assess safety, pharmacokinetics, and effects on circulating estrogens in women with advanced hormone-positive breast cancer. Proc ASCO 2014.

Website(s) or other Internet site(s)
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Expert Opinion piece in Oncology PracticeUpdate http://www.practiceupdate.com/journalscan/9370 or http://prac.co/j/5960d32c-988b-423e-ba24-14ca5c8cc39a?elsca1=soc_share-this acknowledgement of federal support –no

Highlight of Cochrane DR et al Breast Cancer Research 2014 in Feb issue of 2014 NATURE REVIEWS CLINICAL ONCOLOGY. acknowledgement of federal support –yes

Inventions, Patents and Licenses: Nothing to report

Reportable Outcomes: Nothing to report

Other Achievements: Nothing to report

References:

Papers:
Dawn R. Cochrane, Sebastian Bernales, Britta M. Jacobsen, Diana M. Cittelly, Erin N. Howe, Nicholas C. D'Amato, Nicole S. Spoelstra, Annie Jean, Paul Jedlicka, Kathleen C. Torkko, Andy Protter, Anthony D. Elias and J. K. Richer. Role of the Androgen Receptor in Breast Cancer and Preclinical Analysis of Enzalutamide. BREAST CANCER RESEARCH 2014 Jan 22;16(1). PMID: 24451109

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Expert Opinion piece in Oncology PracticeUpdate http://www.practiceupdate.com/journalscan/9370 or http://prac.co/j/5960d32c-988b-423e-ba24-14ca5c8cc39a?elsca1=soc_share-this acknowledgement of federal support –no

Highlight of Cochrane DR et al Breast Cancer Research 2014 in Feb issue of 2014 NATURE REVIEWS CLINICAL ONCOLOGY. acknowledgement of federal support –yes

Appendices

Please see following 4 pages.

Enzalutamide plus exemestane: a pilot study to assess safety, pharmacokinetics, and effects on circulating estrogens in women with advanced hormone-positive breast cancer

Lee S. Schwartzberg,[1] Denise A. Yardley,[2,3] Anthony Elias,[4] Manish R. Patel,[5] Ayca Gucalp,[6] Howard A. Burris,[2,3] Amy C. Peterson,[7] Alison L. Hannah,[7] Martha E. Blaney,[7] Jackie Gibbons,[7] Iulia Cristina Tudor,[7] Joyce L. Steinberg,[8] Patricia LoRusso,[9] Jeffrey R. Infante,[2,3] Clifford A Hudis,[6] and Tiffany A. Traina[6]

[1]The West Clinic, Memphis, TN; [2]Sarah Cannon Research Institute, Nashville, TN; [3]Tennessee Oncology, PLLC, Nashville, TN; [4]Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO; [5]Florida Cancer Specialists and Research Institute, Sarasota, FL; [6]Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; [7]Medivation Inc. San Francisco, CA; [8]Astellas Inc., Northbrook, IL; [9]Department of Oncology, Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI

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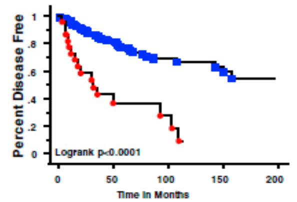
BACKGROUND

Androgen Receptor Signaling in ER/PgR+ Breast Cancer

- Estrogen receptor (ER)/progesterone receptor (PgR)+ breast cancer accounts for > 60% of metastatic breast cancer
  - Advanced disease remains incurable, and some treatments have significant side effects
- The androgen receptor (AR) is expressed in ≥ 75% of patients with ER/PgR+ breast cancer[1,2]
- Almost all ER/PgR+ patients treated with endocrine therapy will eventually develop resistance
- AR expression has been associated with resistance to endocrine therapy[3]
  - Elevated transcript levels of AR were found in patients not responding to tamoxifen[4]
  - Transfection of AR into ER+ cells conferred resistance to both aromatase inhibitor (AI) therapy and tamoxifen[4,5]
- High AR to ER ratios have also been associated with shorter disease-free survival (**Figure 1**)[6]
- ER+ cell lines can be growth stimulated by androgens[7]
  - AR blockers can inhibit this androgen-mediated stimulation
- Aromatase inhibitors block the conversion of androgens to estrogens, resulting in increased androgens (**Figure 2**)

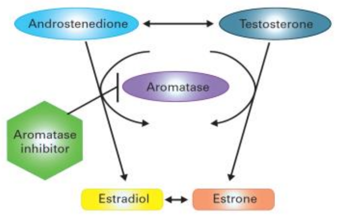
Figure 1. High AR:ER Ratio Correlates with Poor DFS



Blue squares = AR:ER < 2.0; Red squares = AR:ER ≥ 2.0
DFS = disease-free survival
Reference: Cochrane, 2014[6]

Note: current image to be re-drawn and size matched to Figure 2

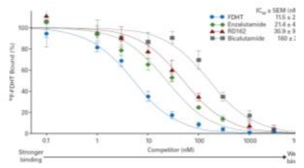
Figure 2. Aromatase Inhibition and Estrogen Synthesis



Enzalutamide is a Potent Inhibitor of AR Signaling

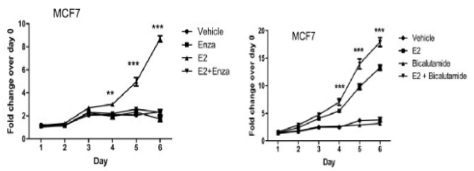
- Enzalutamide directly binds the AR[8] and has been shown to improve survival in men with advanced prostate cancer in post-docetaxel[9] and chemotherapy-naïve[10] patients
- Enzalutamide has 8-fold higher binding affinity for AR than bicalutamide[8] (**Figure 3**)
- Bicalutamide enhanced estradiol-mediated growth of MCF7, whereas enzalutamide inhibited estradiol-mediated growth[6] (**Figure 4**)

Figure 3. Inhibition of AR Binding



FDHT=18F-16β-fluoro-5α-dihydrotestosterone; IC_{50}=inhibitory concentration; SEM = standard error of the mean
Reference: Tran, 2009[8]

Figure 4. Effects of Enzalutamide and Bicalutamide on E2-Mediated Proliferation of Breast Cancer Cells In Vitro.



Reference: Cochrane, 2014[6]

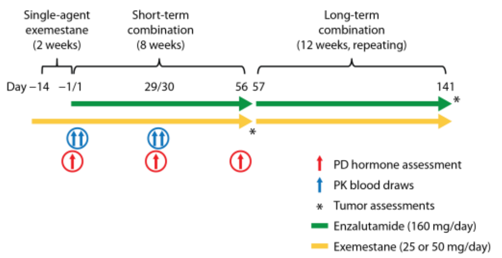
Note: current image to be re-drawn in high resolution and in color

Enzalutamide, Aromatase Inhibitors, and CYP3A4

- Enzalutamide is a strong cytochrome P450 3A4 (CYP3A4) inducer; all AIs are metabolized by CYP3A4
  - Enzalutamide was previously shown to reduce mean exposure to both anastrozole (1 mg) and exemestane (25 mg) at day 29[11]
  - The US Package Insert for exemestane recommends increasing the dose to 50 mg in patients concomitantly receiving a strong CYP3A4 inducer[12]

STUDY DESIGN

Figure 5. Enzalutamide Combined with 25 mg or 50 mg Exemestane (NCT01597193)



Objectives

- Evaluate the safety of enzalutamide combined with exemestane 25 mg or 50 mg
- Evaluate the PK and PD effect of the combination of enzalutamide and exemestane 25 mg or 50 mg

Key Eligibility Criteria

- Must have ER+/PgR+ disease (and be able to provide tissue or pathology documentation)
- Must be able to start or continue therapy with exemestane
- Prior exemestane use and non-measurable disease were allowed

RESULTS

Table 1. Baseline Demographics and Disease Characteristics for Enzalutamide + Exemestane Dose Groups

	Enzalutamide + Exemestane	
	Exemestane 25 mg (N=16)	Exemestane 50 mg (N=23)
Median years in age (range)	56 (42-79)	65 (34-75)
ECOG PS = 1, n (%)	8 (50)	10 (44)
Prior agents for aBC, median (range)	TBC	TBC
Anastrozole/letrozole, n(%)	15 (94)	19 (83)
Exemestane, n(%)	4 (25)	16 (70)
Cytotoxic, n(%)	14 (88)	18 (78)
Prior neoadjuvant/adjuvant, n (%)	9 (56)	17 (74)
Median months from aBC to study entry (range)	27 (9-93)	30 (1-333)
Number of metastatic sites >3, n (%)	1 (6)	3 (13)
Bone metastasis, n (%)	12 (75)	18 (78)
Bone only metastatic disease, n (%)	4 (25)	3 (13)
Visceral disease, n (%)	8 (50)	15 (65)
Measurable disease, n (%)	9 (56)	13 (57)
AR+[a], n/N[b] (%)	7/9 (78)	8/16 (50)

[a] ≥10% nuclear staining on immunohistochemistry; [b] N represents the number of patients with tissue analyzed.
aBC=advanced breast cancer; ECOG=Eastern Cooperative Oncology Group; PS=performance status

Note: all tables will be shaded purple with light/dark shading for alternate rows

Adverse Events Appear Similar Between the Two Dose Cohorts

Table 2. Adverse Events with Enzalutamide in Combination with Exemestane

	All grades of drug-related AEs in ≥3 patients		Any AE grade ≥3 in ≥2 patients	
AE, n (%)	Enza + Exe 25 mg (N=16)	Enza + Exe 50 mg (N=23)	Enza + Exe 25 mg (N=16)	Enza + Exe 50 mg (N=23)
Fatigue	8 (50)	11 (48)	1 (6)	1 (4)
Nausea	7 (44)	9 (39)	0	0
Decreased appetite	4 (25)	4 (17)	0	0
Vomiting	3 (19)	5 (19)	0	0
Hot flash	2 (13)	3 (13)	0	0
Insomnia	2 (13)	1 (4)	0	0
Cognitive disorder[a]	3 (19)	0	0	0
Constipation	1 (6)	2 (9)	0	0
Weight decreased	3 (19)	1 (4)	0	0
Gastroesophageal reflux disease	1 (6)	2 (9)	0	0
Hyperglycemia	2 (13)	1 (4)	0	0
Hypokalemia	0	1 (4)	1 (6)	1 (4)
Hypertension	0	0	0	2 (9)
Neutropenia	2 (13)	0	2 (13)	0
Seizure	None reported			

[a]All grade 1 and attributed to AI but no treatment given
AE=adverse event; Enza = enzalutamide; Exe=exemestane.

Exemestane Exposure When Administered in Combination with Enzalutamide

Figure 6. Mean Plasma Concentration-Time Profiles for Exemestane 25 mg Alone and Exemestane 50 mg with Enzalutamide

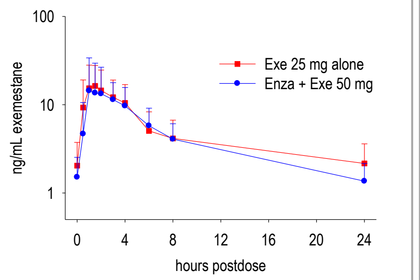


Figure 7. AUC for Exemestane 25 mg Alone and Exemestane 50 mg with Enzalutamide

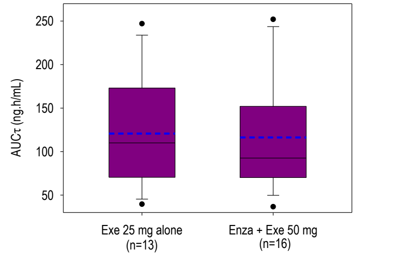


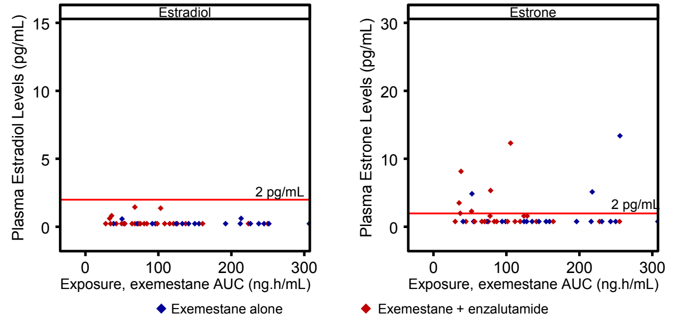
Table 3. AUC Values for Exemestane Alone and Exemestane with Enzalutamide

	AUCτ, ng.h/mL	
Treatments	Exe 25 mg	Exe 50 mg
Exe alone	129 ± 64.8	307 ± 191
Enza + Exe	69.1 ± 37.5	116 ± 67.8
Comparisons	Geometric mean ratios (90% confidence intervals)	
Enza + Exe vs Exe alone	0.575 (0.500, 0.661)	0.434 (0.364, 0.519)
Enza + Exe 50 mg vs Exe 25 mg alone	0.949 (0.650, 1.39)	

- Enzalutamide treatment reduced plasma exposure to exemestane (AUCτ) by ≈ 50%
- Enzalutamide combined with exemestane 50 mg achieved exemestane exposure similar to that of exemestane 25 mg alone. The PK profiles of the two treatments were nearly identical
- Based on these results, doubling the dose of exemestane from 25 mg to 50 mg when combining with enzalutamide provides approximate AUC equivalence to exemestane at 25 mg

Estrogen Inhibition When Enzalutamide is combined with Exemestane

Figure 8. Plasma estradiol and estrone levels in patients treated with exemestane 25 mg or 50 mg alone (day -1) and in combination with enzalutamide (day 29)



Note: shapes to be updated to match those in Fig 6

Red line represents expected estrogen levels with exemestane use

- Estradiol inhibition is maintained when exemestane is combined with enzalutamide (day 29)
- Estrone levels above expected values were observed in patients with lower exemestane AUC

Prior Treatment and Hormone Staining in Patients on Study for ≥16 weeks

Table 4. Prior Treatment and Nuclear Steroid Expression Patterns in Patients on Study ≥ 24 and ≥16 weeks

Cohort	# prior hormones	# prior non-hormones	ER	PgR	AR%	Days on study	
Exe 25 mg	1	3	Medium	Medium	75	450	On study ≥24 weeks
Exe 50 mg	4	6	High	Medium	Unknown	268	
Exe 50 mg	5	5	High	Low	5	267	
Exe[a] 50 mg	1	2	Low	None	30	239	
Exe 50 mg	1	0	High	None	0	210	
Exe[b] 50 mg	3	3	Medium	None	0	187	
Exe 50 mg	4	8	Unknown	Unknown	65	178	
Exe 50 mg	2	1	High	High	10	171	
Exe 25 mg	2	1	High	None	90	156	On study ≥16 weeks
Exe[b] 50 mg	1	6	High	High	Unknown	154	
Exe 50 mg	0	0	High	None	10	152	
Exe 25 mg	1	0	High	None	Unknown	114	

Red font indicates active patient as of 18 APRIL, 2014. High ≥ 67%, medium = 33%–67%, low ≤ 33%
[a]Patient switched from anastrozole to 50 mg exemestane; [b]Previously treated with exemestane 25mg

CONCLUSIONS

- This study defined a 50-mg dose of exemestane for use in combination with standard dose enzalutamide 160 mg/day
  - No apparent increase in frequency or severity of adverse events with 50 relative to 25 mg exemestane
- Reduction of exemestane AUC by enzalutamide can be overcome by doubling the exemestane dose from 25 mg to 50 mg
  - Approximate AUC equivalence was observed between exemestane 50 mg plus enzalutamide and exemestane 25 mg alone
- Exemestane AUC appears to be an important driver of estrogen concentrations
  - Estrogen inhibition was generally maintained when exemestane was combined with enzalutamide
  - Estrone levels above expected values were generally observed in patients with a lower exemestane AUC
- 31% (12 of 39) of patients were on study for ≥16 weeks without progression
- A phase 2, double-blind, randomized study is underway comparing enzalutamide plus exemestane (50 mg/day) to single-agent exemestane (25 mg/day) in patients with advanced ER/PgR+ breast cancer[13] (ASCO poster TPS653)

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ACKNOWLEDGEMENTS

Enzalutamide is co-developed by Medivation, Inc. and Astellas. This study was funded by Medivation, Inc. Editorial assistance funded by Medivation, Inc. and Astellas was provided by Timothy Lohret, PhD from Infusion Communications.

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Poster TPS653 (Yardley)

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Poster TPS1144 (Traina)

MDV3100-08: A Phase 1, Open-Label, Dose-Escalation Study Evaluating the Safety, Tolerability, and Pharmacokinetics of Enzalutamide (Formerly MDV3100) in Patients With Incurable Breast Cancer

18B

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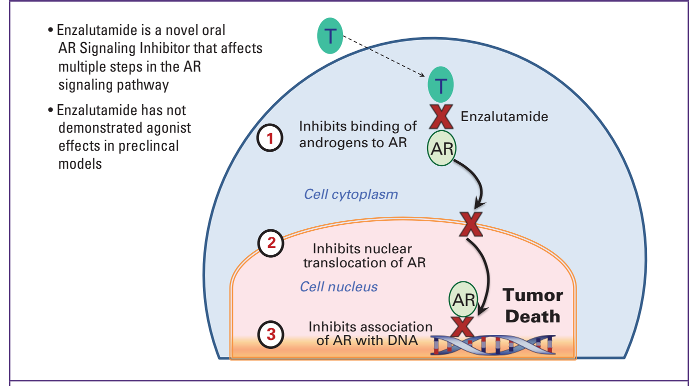
INTRODUCTION AND BACKGROUND

- Breast cancers are biologically diverse in their clinical and gene expression phenotypes.
  - Distinct subtypes are classified by the presence of estrogen (ER), progesterone (PgR), and human epidermal growth factor 2 (HER2) receptors.[1,2]
- 77% of invasive breast tumors across all phenotypes express the androgen receptor (AR)[3]; however, the exact role of AR expression in breast cancer remains largely unknown.
  - AR signaling in ER+ disease
    - AR expression was associated with improved disease-free survival in ER+ cancers.[4]
    - AR expression has been implicated as a mechanism of resistance to tamoxifen in ER+ patients.[5]
    - Aromatase inhibition in ER+ cells transfected to express both aromatase and AR resulted in *stimulated growth*, suggesting a role for combined AR and aromatase inhibition.[6]
  - AR signaling in HER2+ disease
    - AR expression has been identified in over 50% of patients with HER2+/ER– disease.[7,8] An evaluation of published microarray data from 3 patient sets demonstrated that AR gene expression levels correlated with HER2 amplification/overexpression.[9]
    - In MDA-MB-453 (ER–/HER2+/AR+) cells, prolonged AR stimulation enhanced HER2/HER3 signaling, and exposure to bicalutamide downregulated this pathway.[9]
  - AR signaling in triple-negative breast cancer (TNBC: ER–/PgR–/HER2–)
    - Molecular profiling has identified as many as 7 subtypes within TNBC, including luminal AR (LAR).
    - Although lacking ER and PgR expression, LAR is heavily enriched in hormonally regulated pathways and expresses higher levels of AR mRNA compared with other subtypes. AR signaling can promote proliferation that is inhibited by antiandrogens.[9-11]
    - Similar to ER+ breast cancer, improved clinical outcomes have been noted in subsets of patients who are AR+ and are either ER– or ER–/PgR–/HER2–.[12]
    - An ongoing phase 2 study (NCT00468715) is evaluating the antiandrogen bicalutamide in patients with ER– breast cancer.[13]
- Taken together, AR inhibition may have a therapeutic role in breast cancer, especially in patients with AR-expressing tumors.[11]

ENZALUTAMIDE

- Enzalutamide (formerly MDV3100) is a novel AR signaling inhibitor (ARSI).
  - In nonclinical studies using castration-resistant prostate cancer (CRPC) models, enzalutamide bound to AR with greater affinity than bicalutamide. In addition, and contrasting with bicalutamide, enzalutamide inhibited AR nuclear translocation and AR-DNA binding **(Figure 1)**, showed no evidence of AR agonism, and induced tumor regression in a bicalutamide-resistant xenograft model.[14,15]
  - In a phase 1–2 study (NCT00510718), enzalutamide showed durable antitumor effects (soft tissue partial response, 22%), and extended times to PSA and radiographic progression in chemo-naive and chemo-experienced advanced prostate cancer patients.[16,17]
  - The phase 3 AFFIRM trial in men with postdocetaxel metastatic CRPC (NCT00974311) showed an overall survival advantage of 4.8 months with enzalutamide vs placebo (hazard ratio, 0.63; $P<0.0001$), secondary endpoints (eg, radiographic progression-free survival, soft tissue response) were also significantly improved with enzalutamide; furthermore, enzalutamide was generally well tolerated in AFFIRM.[18]

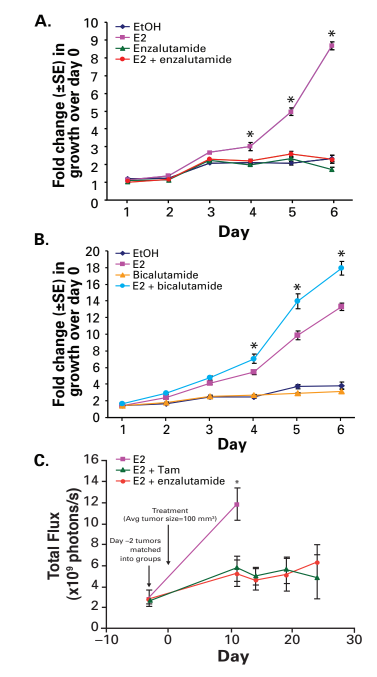
Figure 1. Effects of Enzalutamide on the AR



AR=androgen receptor; T=testosterone.

- Nonclinical studies suggest that enzalutamide blocks androgen- and estradiol-mediated tumor growth in ER+/AR+ breast cancer cell lines **(Figure 2)**[19,20] and blocks androgen-mediated growth in ER–/AR+ cell lines **(Figure 3)**.[19,21]
- Based on the above, this open-label, dose-escalation, phase 1 study is being conducted in patients with incurable breast cancer.

Figure 2. In ER+/AR+ Tumors, Enzalutamide Inhibits E2-Mediated Growth in MCF7 Cells (A), Whereas Bicalutamide Does Not (B), and Enzalutamide Suppresses E2-Mediated Growth as Effectively as Tamoxifen In Vivo (C)



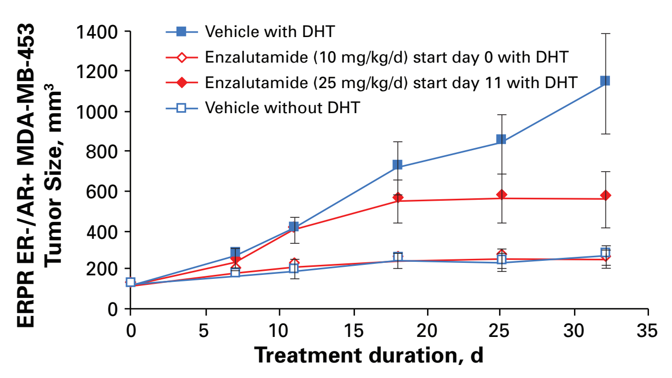
Proliferation assays were performed on MCF7 cells treated with vehicle control (EtOH), 10 nM E2, 10 μM enzalutamide, or a combination of E2 and enzalutamide. $^{*}P<0.05$; 2-way analysis of variance with Bonferroni posttest for E2 vs E2 + enzalutamide.

MCF7 cells were plated (6 wells/time point) in phenol-red free, charcoal-stripped serum media; after 24 hours, they were treated with vehicle (EtOH), 10 nM E2, 1 mM bicalutamide, or the combination of E2 + bicalutamide and measured using MTT assays. $^{*}P<0.05$; 2-way analysis of variance with Bonferroni posttest for E2 vs E2 + bicalutamide.

1×10^6 luciferase-expressing MCF7 cells were mixed with 100 μL of matrigel and injected bilaterally into the fourth mammary glands of 6- to 8-week-old ovariectomized nude mice, and E2 pellets implanted subcutaneously. Tumor burden was measured by whole-body in vivo luminescent imaging. When tumors reached an average of 100 mm^3, mice were matched into 3 groups based on tumor burden, with 1 group receiving control chow, 1 group receiving control chow and a tamoxifen pellet implanted subcutaneously, and the third group receiving chow containing 50 mg/kg enzalutamide. Day 11 is the last day shown for the E2-only treated mice because signal saturation was reached by the next imaging. $^{*}P<0.01$ for E2 vs E2 + enzalutamide and E2 vs E2 + Tam

AR=androgen receptor; E2=estradiol; ER=estrogen receptor; MCF7=Michigan Cancer Foundation–7 (human breast adenocarcinoma cell line); tam=tamoxifen.

Figure 3. Enzalutamide Blocks DHT-Mediated Growth in ER–/AR+ Breast Cancer Xenografts



AR=androgen receptor; DHT=dihydrotestosterone; ER=estrogen receptor; IL2Rgc–/–=IL-2 receptor gamma chain deficient; MDA-MB-453=apocrine human breast adenocarcinoma cell line; NOD/SCID=nonobese diabetic severe combined immunodeficient.

Cells (1×10^6) in matrigel were injected into the fourth inguinal mammary glands of NOD-SCID-IL2Rgc–/– 5- to 6-week-old mice implanted with either vehicle (cellulose) or DHT pellets (12.5 mg 60-day-release pellets) subcutaneously (back of neck). Treatment was initiated on either day 0 (when tumors were 100 mm^3) with 12.5 mg/kg/d enzalutamide by oral gavage, or day 11 when tumors were 400 mm^3, with 25 mg/kg/d enzalutamide. Tumors were measured by caliper and mean volumes ± SEM, n=10/group.

OBJECTIVES

- The primary objectives are to (1) evaluate the safety, tolerability, and dose-limiting toxicities (DLTs) of enzalutamide in patients with incurable breast cancer, and (2) evaluate the safety and tolerability of daily enzalutamide at the recommended phase 2 dose in patients with incurable AR+ breast cancer.
- The secondary objective is to characterize the pharmacokinetic (PK) properties of enzalutamide.
- Exploratory objectives include preliminary assessment of enzalutamide antitumor activity in patients with incurable breast cancer and assessment of the extent of hormone receptor expression and signaling (including AR, ER, PgR) in tumor tissue and evaluation of the relationship of this expression with enzalutamide activity.

METHODS

Study Design and Conduct

- The study opened April 2012 and has an expected enrollment of 27 patients from 3 participating sites
  - Karmanos Cancer Institute (Detroit, MI)
  - University of Colorado Cancer Center (Aurora, CO)
  - Memorial Sloan-Kettering Cancer Center (New York, NY)
- There are 2 stages to the study (**Figure 4**):
  - Dose-escalation (stage 1) evaluating at least 2 dose levels in ~12 patients to identify the phase 2 dose
  - Dose-expansion (stage 2) in ~15 patients treated at the phase 2 dose

Patients

- Stage 1 will comprise patients with incurable breast cancer that has progressed despite at least 2 systemic treatments.
- Stage 2 will be further limited to patients with incurable AR+ breast cancer **(Table 1)**.

Table 1. Key Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Stage 1 • Patients ≥18 y of age • Histologically confirmed breast cancer • Received ≥2 lines of cytotoxic therapy in the advanced setting (for patients with HER2– with ER+ and/or PgR+ cancer, this may be 2 lines of endocrine therapy; patients with ER+/PgR+ disease must be postmenopausal) • ECOG performance status of 0 or 1; patients with stable ECOG 2 (≥2 mo) may be allowed • Life expectancy ≥3 mo Stage 2 • All of the above and – Must have AR+ breast cancer*	• Severe concurrent disease, infection, or comorbidity • Pregnancy or lactation • Known/suspected brain metastases or leptomeningeal disease • History of other malignancies within 5 y • Palliative radiation therapy to bony metastases ≤2 wk of study therapy initiation • History of seizure or treatment with antiseizure medication or loss of consciousness ≤1 y before visit 1 • Previous use of or participation in a clinical trial of any agent that blocks androgen synthesis or targets the AR

AR=androgen receptor; ECOG=Eastern Cooperative Oncology Group; ER=estrogen receptor; HER=Human Epidermal Growth Factor Receptor; PgR=progesterone receptor.

*Defined as ≥10% of tumor cells with nuclear AR staining by immunohistochemistry (enrollment may be based on the local pathologist's findings; however, tissue must be forwarded to a central pathology laboratory for confirmation).

Study Treatments

- Stage 1 has 5 treatment periods:
  - Period 1: Screening
  - Period 2: Single-dose PK period lasting 7 days; patients will receive oral enzalutamide on study day 1 only, with serial PK sampling through day 8
  - Period 3: Short-term dosing period consisting of 49 days of consecutive enzalutamide at assigned dosing level; tumor assessments will be performed at the end of this period
  - Period 4: Long-term dosing period is 12 weeks of consecutive dosing at assigned dose level and may be repeated based on tumor assessments that will occur every 84 days and ongoing assessments of tolerability
  - Period 5: Safety follow-up
- Stage 2 has 4 periods that match stage 1 periods 1, 3, 4, and 5.

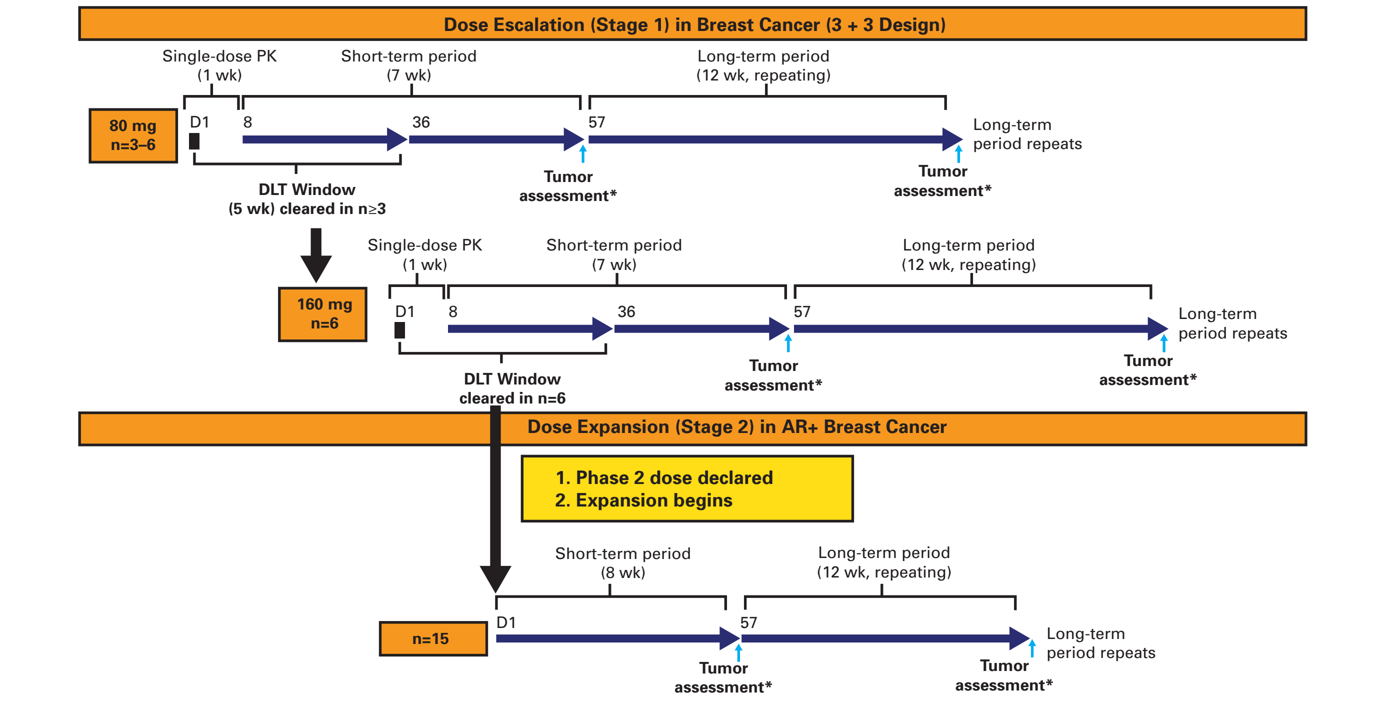
Assessments

- Adverse event assessment, vital signs, laboratory evaluations, physical examinations
- PK analysis (collections at multiple time points in stage 1 and 2)
- Tumor assessment and documentation of disease will be performed after the first 7 weeks and then every 12 weeks thereafter in both stage 1 and 2.

Statistical Methods and Endpoints

- Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and severity assessed (National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03).
- PK analyses will include mean plasma concentration vs time data for enzalutamide after single- and multiple-dose administration.
  - Noncompartmental methods will assess PK parameters of maximum observed concentration (C_{max}), time to C_{max}, area under the concentration vs time curve, terminal half-life, apparent oral clearance, apparent volume of distribution, peak-to-trough, dose proportionality, and accumulation.

Figure 4. Study Design



AR=androgen receptor; DLT=dose-limiting toxicity; PK=pharmacokinetics. *Patients may continue if no disease progression.

- There is no formal plan to evaluate efficacy
  - Overall response: objective response in patients with measurable disease is defined per RECIST version 1.1[22] and will be confirmed by repeat assessment after ≥4 weeks.
- Nuclear AR expression will be measured using immunohistochemistry, and the relationship between tumor response and degree of AR expression and/or AR signaling will be examined.

CONCLUSIONS

- This will be the first study that examines the safety, tolerability, DLTs, and PK of enzalutamide in patients with incurable breast cancer. Measures of efficacy are exploratory.

Acknowledgments

Enzalutamide is being co-developed by Medivation, Inc., and Astellas. Funding was provided by both for poster development; assistance with poster development was provided by Kris Schuler, MS, from Complete Healthcare Communications, Inc.

The information concerns an investigational use of a drug that has not been approved by the US Food and Drug Administration or the European Medicines Agency.

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San Antonio Breast Cancer Symposium – Cancer Therapy and Research Center at UT Health Science Center – December 10–14, 2013

Poster PD3-6

A Phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of enzalutamide (previously MDV3100) alone or in combination with an aromatase inhibitor in women with advanced breast cancer

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Poster OT3-2-08 (Traina)

Poster OT3-2-01 (Yardley)

BACKGROUND

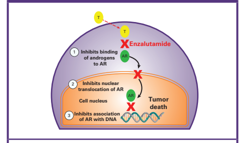
The androgen receptor in breast cancer

- Enzalutamide (enza) is a potent androgen receptor (AR) inhibitor[1] that has been approved for the treatment of men with metastatic castration-resistant prostate cancer (mCRPC) who have previously received docetaxel (**Figure 1**).[2]
- The Phase 3 PREVAIL trial evaluating enza in chemotherapy-naive patients with advanced prostate cancer met both co-primary end points of overall survival and radiographic progression-free survival.
- The AR is expressed in 70% of all breast cancers and across all subtypes: estrogen receptor-positive (ER+), triple-negative breast cancer (TNBC), and human epidermal growth factor receptor 2-positive (HER2+).[3]

Estrogen synthesis and relevance to breast cancer

- Androgens are converted by an enzyme, aromatase, to estrone and estradiol (**Figure 2**).
- Aromatase inhibitors (AIs) block the conversion of androgens to estrogens by inhibiting aromatase, resulting in a concomitant increase in androgens.
  - Enza may add to the activity of AIs by blocking potential growth stimulation of the AR due to increased circulating androgens.
  - Enza is a potent CYP3A4 inducer. Both exemestane (exe) and anastrozole (ana) are metabolized by CYP3A4; stage 2 of this trial investigated whether any observed drug-drug interaction between enza and these AIs would translate into an effect on circulating estrogens.

Figure 1. Enzalutamide MOA



AR=androgen receptor; T=testosterone.

Figure 2. Estrogen synthesis

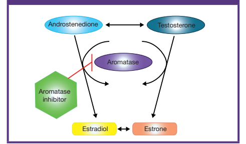


Table 1. Data supporting AR signaling in breast cancer subtypes

	ER+/PgR+ BrCa	AR+ TNBC	HER2+ BrCa
AR expression in patient tumor samples	86%[3]	10-30%[3,4]	~60%[5]
Preclinically, tumor growth stimulated by androgens	Yes (MCF7)[6]	Yes (MDA-MB-453)[6,7]	Yes (BT474)[6,7]
Preclinically, tumor growth blocked by enza	Yes[6]	Yes[6,7]	Yes[6]
Other	• Preclinically, overexpression of AR conferred resistance to tamoxifen or ana[8,9] • Clinically, an AR:ER ratio >1.3 predicted for shorter DFS following adjuvant tamoxifen[10]	• 19% clinical benefit rate from bicalutamide in pts with AR+ TNBC[4] • The Luminal AR (LAR) subtype expresses 9 fold higher levels of AR and is heavily enriched in hormonally regulated pathways[7]	• Preclinically, the combination of enza + trastuzumab inhibited tumor cell growth more effectively than either agent alone[6]

Ana=anastrozole; AR=androgen receptor; BrCa=breast cancer; DFS=disease-free survival; enza=enzalutamide; ER=estrogen receptor; HER2+=human epidermal growth factor receptor-2 positive; PgR=progesterone receptor; TNBC=triple-negative breast cancer.

OBJECTIVES

- Stage 1: To determine the pharmacokinetics, safety, and tolerability of enza in women with breast cancer and to identify the recommended dose for further testing.
- Stage 2: To determine the effects of enza on the pharmacokinetics, pharmacodynamics, safety, and tolerability of either ana (1 mg) or exe (25 mg).

PATIENT ELIGIBILITY

Key criteria

All stages

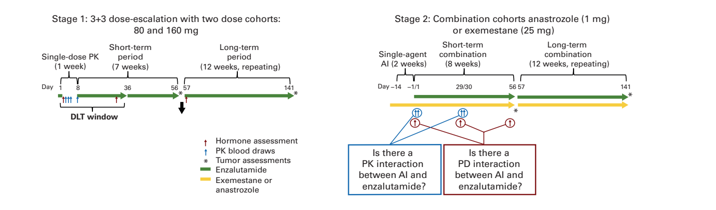
1. Women with advanced breast cancer (aBC) with ECOG performance status 0–2
2. Received ≥2 lines of therapy for aBC (not required for AI cohorts in stage 2) which may have been two prior hormones for ER+/PgR+ advanced disease
3. ER+/PgR+ patients must be postmenopausal
4. Adequate kidney, liver, and bone marrow function
5. No known or suspected brain metastasis or active leptomeningeal disease
6. No history of seizure or any condition that may predispose to seizure

Stage 2 (additional criteria)

1. Must have ER+/PgR+ disease (and be able to provide tissue or pathology documentation)
2. Must be able to start or continue therapy with either ana or exe

STUDY DESIGN

Figure 3. Study design



AI=aromatase inhibitor; DLT=dose-limiting toxicity; PD=pharmacodynamics; PK=pharmacokinetics.

RESULTS

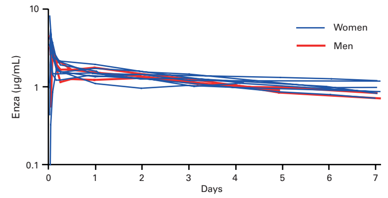
Table 2. Baseline patient demographics and characteristics

	Stage 1: Enza only		Stage 2: Enza + AI	
	80 mg (N=7)	160 mg (N=8)	Anastrozole (N=20)	Exemestane (N=16)
Median (range) age, years	56 (46–69)	59 (40–71)	62 (30–84)	56 (42–79)
ECOG performance status, n (%)				
1	6 (86)	7 (88)	5 (25)	8 (50)
Number of prior agents for aBC (median, range)				
Hormonal	3 (0–4)	1 (0–3)	2 (0–5)	2 (0–3)
Non-hormonal	7 (0–12)	4 (0–10)	2 (0–10)	2 (0–5)
Prior neoadjuvant/adjuvant, n (%)	5 (71)	8 (100)	14 (70)	9 (56)
Months from dx of aBC to study entry (median, range)	45 (22–123)	45 (11–104)	49 (2–125)	33 (9–97)
Number of metastatic sites >3, n (%)	4 (57)	0	1 (5)	0
Bone metastasis, n (%)	5 (71)	7 (88)	16 (80)	12 (75)
Visceral disease, n (%)	6 (86)	3 (38)	13 (65)	8 (50)
Measurable disease, n (%)	1 (14)	4 (50)	11 (55)	9 (56)
Breast cancer subtype, n (%)[a]				
ER+/PgR+	6 (86)	5 (63)	20 (100)	16 (100)
HER2 amplified	1 (14)	1 (13)	0	1 (6)
Triple negative	1 (14)	3 (38)	0	0
AR+, n/N (%)[b]	4/7 (57)	2/5 (40)	10/14 (71)	9/11 (82)

[a]Investigator-defined; [b]≥10% nuclear staining on immunohistochemistry; N represents the number of patients with tissue analyzed.
aBC=advanced breast cancer; AI=aromatase inhibitor; AR=androgen receptor; ECOG=Eastern Cooperative Oncology Group; enza=enzalutamide; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; PgR=progesterone receptor.

Stage 1: Enzalutamide 160 mg/day, with or without food, is the recommended dose for both women and men

Figure 4. Pharmacokinetics of enzalutamide are similar in women with breast cancer versus men with prostate cancer



	Women (n=15)	Men (n=30)
CL/F (L/h)	0.42 (0.16–0.84)	0.56 (0.33–1.02)
Vz/F (L)	121 (54–269)	110 (70–177)
$t_{1/2}$ (days)	9.6 (2.6–23.0)	5.8 (2.8–10.2)

Enzalutamide PK profile in breast cancer patients (women) after a single oral dose of 160 mg versus prostate cancer patients (men) after a single oral dose of 150 mg (dose normalized to 160 mg). PK data from men are from a Phase 1–2 dose-escalation study (S-3100-1-01) in which PK was determined to be dose-linear over the dose range of 30–360 mg. Table displays mean (range) observed PK parameters.
CL/F= apparent oral clearance; enza=enzalutamide; PK=pharmacokinetic; $t_{1/2}$=terminal half-life for women after a single oral dose of 80 mg (n=7) or 160 mg (n=8) and for men after a single oral dose of 30–360 mg (n=30); Vz/F=apparent volume of distribution.

- The safety and tolerability profile of enza in women appears to be similar to that observed in men (**Table 3**).

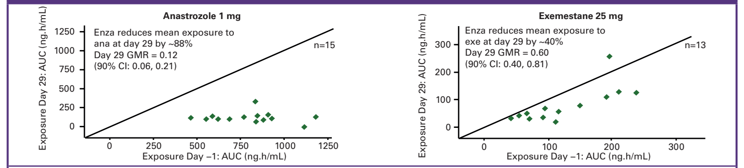
Table 3. Adverse events with single-agent enzalutamide

AE, n (%)	All grades of drug-related AEs in ≥2 patients		OR	Any AE grade ≥3 in ≥1 patient	
	80 mg (N=7)	160 mg (N=8)		80 mg (N=7)	160 mg (N=8)
AST increased	3 (43)	1 (13)		1 (14)	0
Nasal congestion	0	3 (38)		0	0
Nausea	1 (14)	2 (25)		0	0
ALP increased	0	2 (25)		0	0
Cough	0	2 (25)		0	0
Dry eye	0	2 (25)		0	0
Weight decreased	0	2 (25)		0	0
Diarrhea	1 (14)	1 (13)		0	0
Anemia	0	1 (13)		1 (14)	1 (13)
INR increased	1 (14)	0		1 (14)	0
Adrenal insufficiency[a]	1 (14)	0		1 (14)[a]	0
Hydronephrosis	0	0		1 (14)	0
Hypertension[b]	0	0		1 (14)	0
Seizure[b]	None reported				

[a]This was also a dose-limiting toxicity observed in a patient with a pre-existing history of adrenal insufficiency who experienced an exacerbation on study drug; [b]AEs of special interest.
AE=adverse event; ALP=alkaline phosphatase; AST=aspartate aminotransferase; INR=international normalized ratio.

Stage 2: Enzalutamide reduces mean exposure to both anastrozole (~90%) and exemestane (~40%)

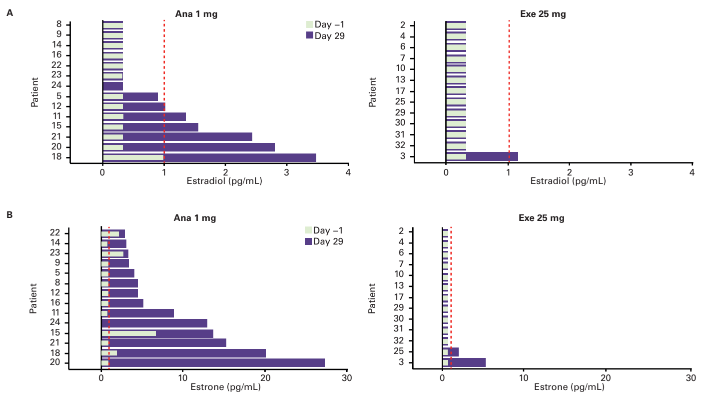
Figure 5. In combination with enzalutamide, AUC decreased by 88% for anastrozole and by 40% for exemestane



Includes only patients with dose interruption or modification of their AI from Day-14 to Day 29.
AUC=area under the curve; CI=confidence interval; GMR=geometric mean ratio.

Aromatase inhibition is maintained with exemestane but variable with anastrozole

Figure 6. Estradiol (A) and estrone (B) levels during exposure to enzalutamide



Includes only patients with no dose interruption or modification of their AI from Day -14 to Day 29. Patient 26 was excluded from analyses for having received exogenous conjugated estrogen.
AI=aromatase inhibitor; ana=anastrozole; exe=exemestane. Dashed line represents expected estrogen levels with anastrozole and exemestane use.[11,12]

Table 4. Adverse events with enzalutamide in combination with anastrozole or exemestane

AE, n (%)	All grades of drug-related AEs in ≥3 patients		OR	Any AE grade ≥3 in ≥2 patients	
	Ana cohort (N=20)	Exe cohort (N=16)		Ana cohort (N=20)	Exe cohort (N=16)
Fatigue	12 (60)	8 (50)		1 (5)	1 (6)
Decreased appetite	10 (50)	4 (25)		0	0
Nausea	9 (45)	7 (44)		0	1 (6)
Hot flash	4 (20)	2 (13)		0	0
Vomiting	3 (15)	3 (19)		0	0
Diarrhea	4 (20)	1 (6)		1 (5)	0
Insomnia	3 (15)	2 (13)		0	0
Cognitive disorder[a]	2 (10)	3 (19)		0	0
Dysgeusia	3 (15)	1 (6)		0	0
Weight decreased	1 (5)	3 (19)		0	0
Back pain	2 (10)	2 (13)		0	0
Anxiety	1 (5)	2 (13)		0	0
Neutropenia[b]	0	2 (13)		0	2 (13)
Seizure[b]	None reported				

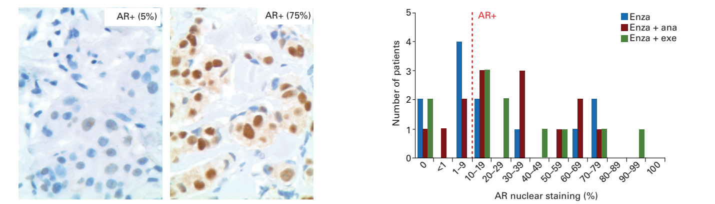
[a]All grade 1 and attributed to AI; no treatment given; [b]AEs of special interest.
AE=adverse event; AI=aromatase inhibitor; ana=anastrozole; exe=exemestane.

Table 5. Patients on study for ≥16 weeks

Stage: cohort	Patient	# prior hormones	# prior non-hormones	ER	PgR	AR%	Days on study	
2: Exe	26	1	3	Medium	Medium	75	304	On study >24 weeks
2: Ana	8	1	0	High	High	30	275	
2: Ana/exe[a]	14	1	2	Low	None	30	242	
2: Exe[b]	25	3	3	Medium	None	0	187	
2: Exe	29	2	1	High	None	90	156	
2: Ana/exe[a]	23	1	6	High	High	Unknown	154	
2: Ana[b]	16	0	0	High	High	60	153	
2: Ana	19	0	0	High	Low	10	152	
2: Ana	12	2	2	High	None	65	152	
1: 160 mg	1	1	1	High	Low	10	141	
1: 80 mg[c]	33	4	8	High	High	75	137	
2: Ana[b]	27	5	2	Positive[d]	Positive[d]	Unknown	126	On study >16 weeks
2: Ana[b]	28	4	3	High	High	Unknown	126	
2: Exe	10	1	0	High	None	20	114	

Red font indicates active patient as of 10/8/13. High = >67%, medium = 33–67%, low = <33%.
[a]Patient switched to 50 mg exe; [b]Previously treated with same AI; [c]HER2 amplified; [d]Tested using automated quantitative analysis.
aBC=advanced breast cancer; AI=aromatase inhibitor; ana=anastrozole; AR=androgen receptor; ER=estrogen receptor; exe=exemestane; HER2=human epidermal growth factor receptor 2; PgR=progesterone receptor.

Figure 7. Androgen receptor staining



Representative AR staining (imaged at 60×). 4 µm formalin-fixed paraffin-embedded sections were cut and stained with anti-AR clone AR441 (Dako) using Clarient CLIA AR immunohistochemistry assay. For determination of AR expression, only nuclear intensity and a proportion of positive staining in invasive tumor cells were evaluated. Samples ≥10% nuclear staining were scored as positive.

Distribution of samples based on nuclear staining. Patients were grouped according to their percentage of AR+ nuclear staining irrespective of their intensities. **Patients whose tissue sample was from bone were excluded from this analysis.**
ana=anastrozole; AR=androgen receptor; enza=enzalutamide; exe=exemestane.

CONCLUSIONS

- Enza, taken daily at a dose of 160 mg, is generally well tolerated as a single agent and in combination with either ana or exe.
  - No seizures have been observed to date.
- Enza can be combined with exe and effective aromatase inhibition is maintained.
  - Due to the observed decrease in exposure the study has been expanded to evaluate 50 mg exe plus enza; data expected in 2014.
- Enza reduced mean exposure to ana by ~88% and the effects on hormones were variable.
- AR expression in this population is representative of that reported in the literature.[3,7]
- 14 of the 36 patients enrolled into the AI cohorts have been on study for 4 months or longer.
- Two global Phase 2 clinical trials are enrolling:
  - Protocol MDV3100-11 (SABCS poster OT3-2-08): single-agent enza in AR+ TNBC (primary end point: clinical benefit rate)
  - Protocol MDV3100-12 (SABCS poster OT3-2-01): a randomized trial investigating exe plus enza versus exe plus placebo in hormone-receptor-positive breast cancer (primary end point: progression-free survival).

ACKNOWLEDGMENTS

Enzalutamide is co-developed by Medivation, Inc. and Astellas. This study was funded by Medivation, Inc. Editorial assistance funded by Medivation, Inc. and Astellas was provided by Hilary Dean from Complete HealthVizion.

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Poster 10P

A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of enzalutamide (previously MDV3100) alone or in combination with an aromatase inhibitor in women with advanced breast cancer

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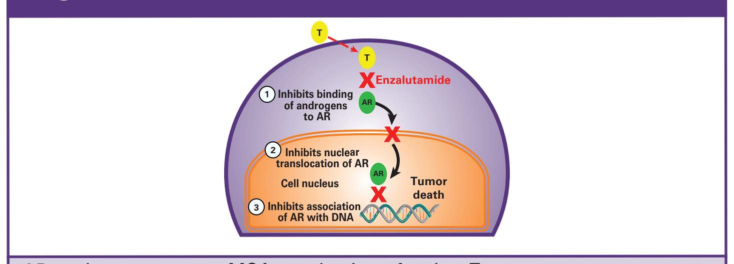
BACKGROUND

The androgen receptor in breast cancer

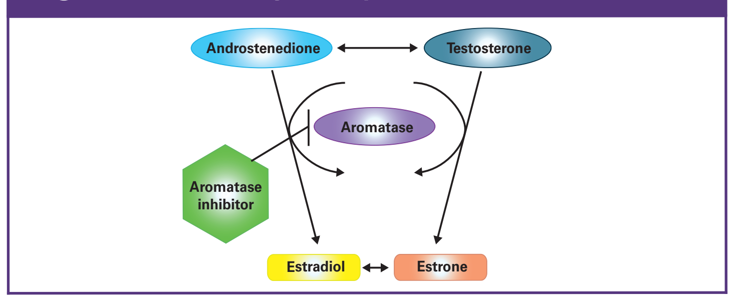
- Enzalutamide (enza) is a potent androgen receptor (AR) inhibitor[1] that has been approved for the treatment of men with metastatic castration-resistant prostate cancer (mCRPC) who have previously received docetaxel (**Figure 1**).[2]
- The Phase 3 PREVAIL trial evaluating enza in chemotherapy-naive patients with advanced prostate cancer met both co-primary endpoints of overall survival and radiographic progression-free survival.
- The AR is expressed in 70% of all breast cancers and across all subtypes: estrogen receptor-positive (ER+), triple-negative breast cancer (TNBC), and human epidermal growth factor receptor 2-positive (HER2+).[3]

Figure 1. Enzalutamide MOA



AR=androgen receptor; MOA=mechanism of action; T=testosterone.

Figure 2. Estrogen synthesis



Estrogen synthesis and relevance to breast cancer

- Androgens are converted by an enzyme, aromatase, to estrone and estradiol (**Figure 2**).
- Aromatase inhibitors (AIs) block the conversion of androgens to estrogens by inhibiting aromatase, resulting in a concomitant increase in androgens.
  - Enza may add to the activity of AIs by blocking potential growth stimulation of the AR due to increased circulating androgens.
  - Enza is a potent CYP3A4 inducer. Both exemestane (exe) and anastrozole (ana) are metabolized by CYP3A4; stage 2 of this trial investigated whether any observed drug-drug interaction between enza and these AIs would translate into an effect on circulating estrogens.

Table 1. Data supporting AR signaling in breast cancer subtypes

	ER+/PgR+ BrCa	AR+ TNBC	HER2+ BrCa
AR in clinical samples, %	86[3]	10–30[3,4]	≈60[5]
Tumor stimulated by DHT	Yes[6]	Yes[6,7]	Yes[6,7]
Tumor inhibited by enza	Yes[6]	Yes[6,7]	Yes[6]
Other	• ↑ expression of AR conferred resistance to endocrine therapy[8,9] • A ↑ AR:ER ratio (≥1.3) conferred worse prognosis following adjuvant tamoxifen[10]	• 19% clinical benefit rate from bicalutamide observed in patients with AR+ TNBC[4] • The LAR subtype expresses ↑ AR and hormonally regulated pathways[7]	• DHT can stimulate growth of tumor cell lines • The combination of enza + tras inhibited tumor growth[6]

AR=androgen receptor; BrCa=breast cancer; DFS=disease-free survival; DHT=dihydrotestosterone; enza=enzalutamide; ER=estrogen receptor; HER2+=human epidermal growth factor receptor 2 positive; LAR=luminal androgen receptor; PgR=progesterone receptor; TNBC=triple-negative breast cancer; tras=trastuzumab.

OBJECTIVES

- Stage 1: To determine the pharmacokinetics, safety, and tolerability of enza in women with breast cancer and to identify the recommended dose for further testing.
- Stage 2: To determine the effects of enza on the pharmacokinetics, pharmacodynamics, safety, and tolerability of either ana (1 mg) or exe (25 mg).

PATIENT ELIGIBILITY

Key criteria

All stages

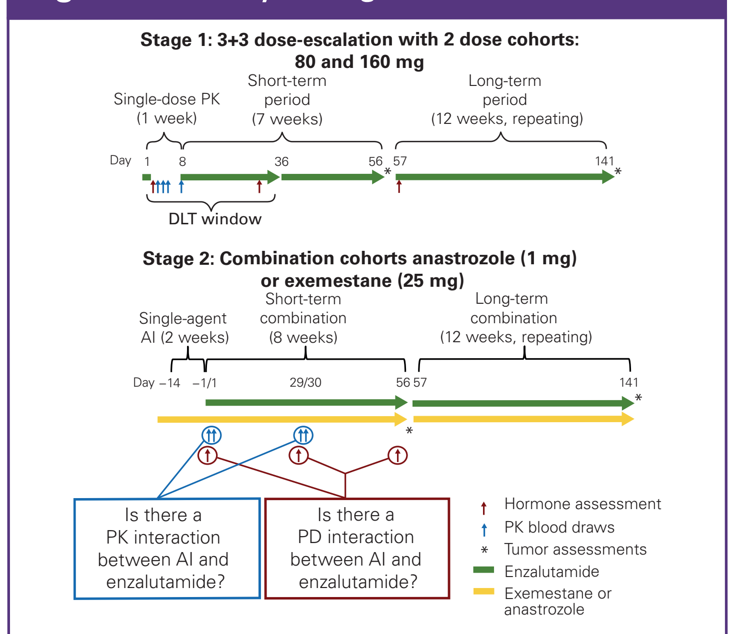
1. Women with advanced breast cancer (aBC) with ECOG performance status grade 0–2
2. Received ≥2 lines of therapy for aBC (not required for AI cohorts in stage 2), which may have been 2 prior hormones for ER+/PgR+ advanced disease
3. ER+/PgR+ patients must be postmenopausal
4. Adequate kidney, liver, and bone marrow function
5. No known or suspected brain metastasis or active leptomeningeal disease
6. No history of seizure or any condition that may predispose to seizure

Stage 2 (additional criteria)

1. Must have ER+/PgR+ disease (and be able to provide tissue or pathology documentation)
2. Must be able to start or continue therapy with either ana or exe

STUDY DESIGN

Figure 3. Study design (NCT01597193)



AI=aromatase inhibitor; DLT=dose-limiting toxicity; PD=pharmacodynamics; PK=pharmacokinetics.

RESULTS

Table 2. Baseline patient demographics and characteristics

	Stage 1: Enza only		Stage 2: Enza + AI	
	80 mg (n=7)	160 mg (n=8)	Ana (n=20)	Exe (n=16)
Years of age, median (range)	56 (46–69)	59 (40–71)	62 (30–84)	56 (42–79)
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[a]Investigator-defined; [b]≥10% nuclear staining on immunohistochemistry; [c]N represents the number of patients with tissue analyzed. aBC=advanced breast cancer; AI=aromatase inhibitor; ana=anastrozole; AR=androgen receptor; dx=diagnosis; ECOG=Eastern Cooperative Oncology Group; enza=enzalutamide; exe=exemestane; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; PgR=progesterone receptor.

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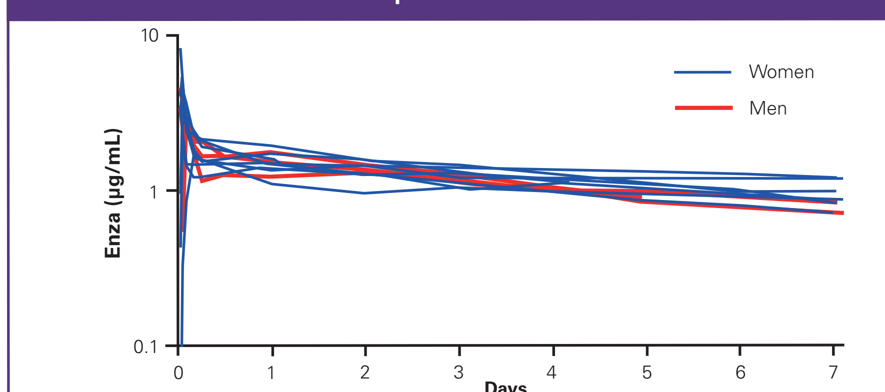

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Diarrhea	1 (14)	1 (13)		0	0
Anemia	0	1 (13)		1 (14)	1 (13)
INR increased	1 (14)	0		1 (14)	0
Adrenal insufficiency[a]	1 (14)	0		1 (14)	0
Hydronephrosis	0	0		1 (14)	0
Hypertension[b]	0	0		1 (14)	0
Seizure[b]	None reported				

[a]This was also a dose-limiting toxicity observed in a patient with a pre-existing history of adrenal insufficiency who experienced an exacerbation on study drug; [b]AEs of special interest. AE=adverse event; ALP=alkaline phosphatase; AST=aspartate aminotransferase; INR=international normalized ratio.

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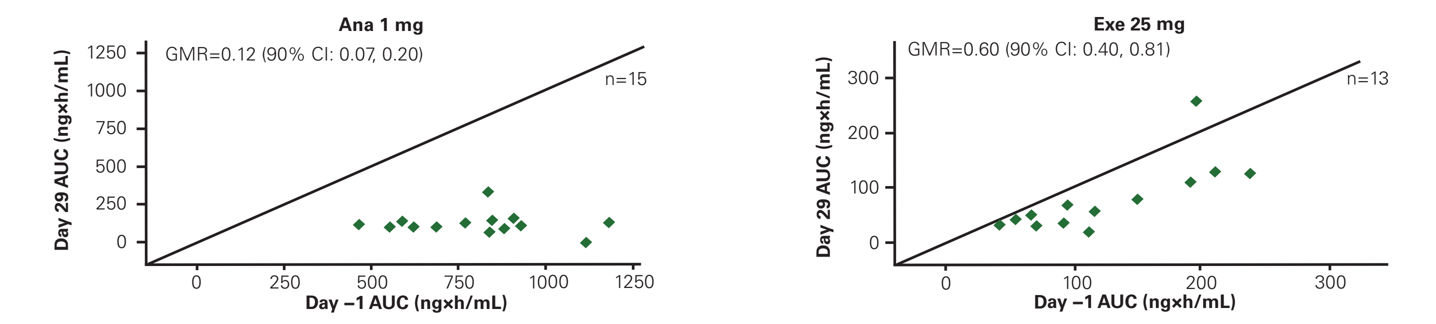

Includes only patients with no dose interruption or modification of their AI from Day –14 to Day 29.
AI=aromatase inhibitor; Ana=anastrozole; AUC=area under the curve; CI=confidence interval; exe=exemestane; GMR=geometric mean ratio (AUC Day 29/AUC Day –1).

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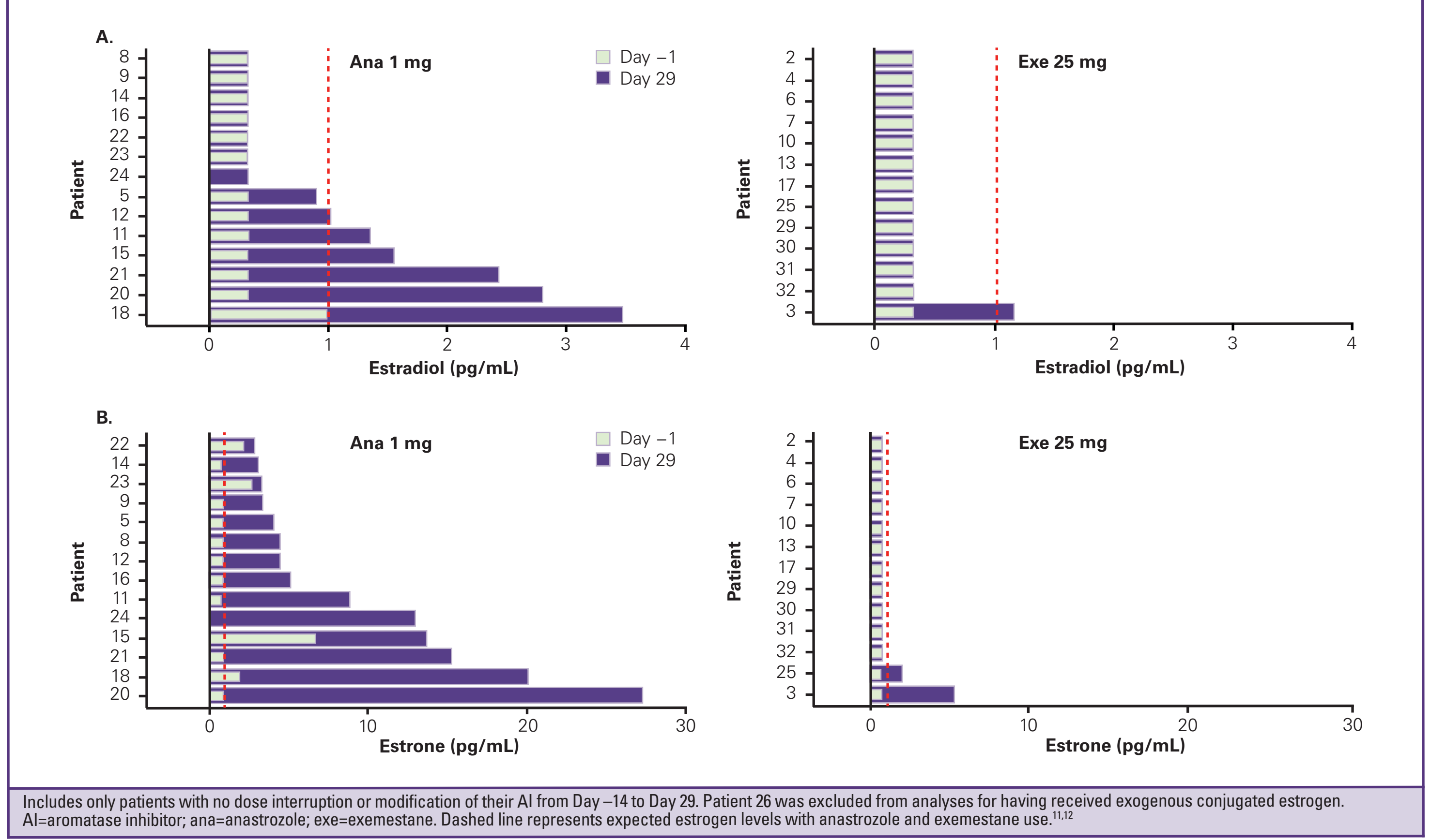

Includes only patients with no dose interruption or modification of their AI from Day –14 to Day 29. Patient 26 was excluded from analyses for having received exogenous conjugated estrogen.
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Decreased appetite	10 (50)	4 (25)		0	0
Nausea	9 (45)	7 (44)		0	1 (6)
Hot flash	4 (20)	2 (13)		0	0
Vomiting	3 (15)	3 (19)		0	0
Diarrhea	4 (20)	1 (6)		1 (5)	0
Insomnia	3 (15)	2 (13)		0	0
Cognitive disorder[a]	2 (10)	3 (19)		0	0
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Weight decreased	1 (5)	3 (19)		0	0
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Stage: cohort	Patient	No. of prior hormones	No. of prior non-hormones	ER	PgR	AR%	Days on study	
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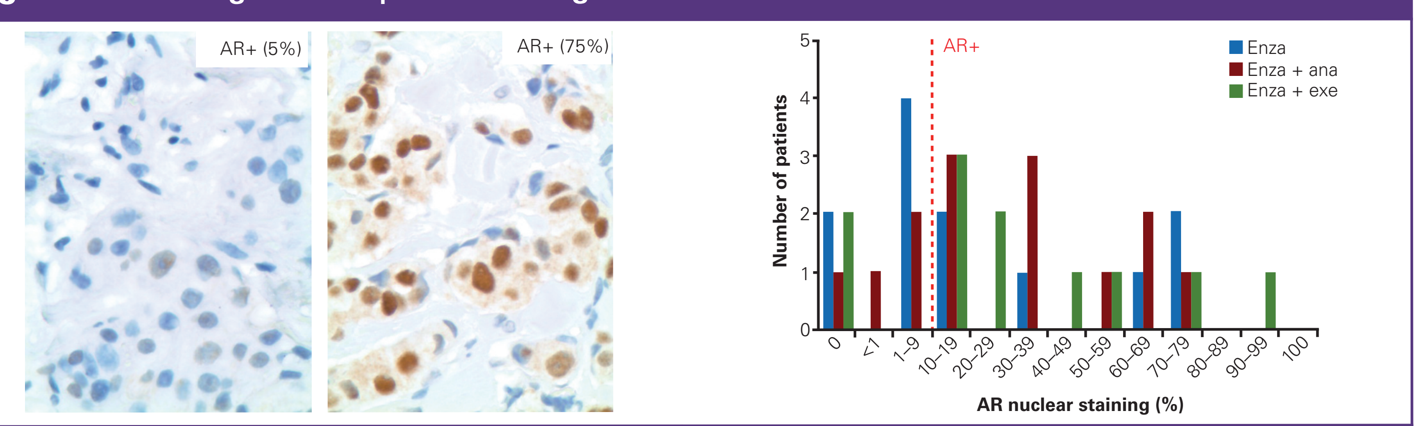
2: Ana	8	1	0	High	High	30	228	
2: Ana/exe[a]	14	1	2	Low	None	30	195	
2: Exe[b]	25	3	3	Medium	None	0	187	
2: Exe	29	2	1	High	None	90	156	On study ≥16 weeks
2: Ana/exe[a]	23	1	6	High	High	Unknown	154	
2: Ana[b]	16	0	0	High	High	60	153	
2: Ana	19	0	0	High	Low	10	152	
2: Ana	12	2	2	High	None	65	152	
1: 160 mg	1	1	1	High	Low	10	141	
1: 80 mg[c]	33	4	8	High	High	75	137	
2: Ana[b]	27	5	2	Positive[d]	Positive[d]	Unknown	126	
2: Ana[b]	28	4	3	High	High	Unknown	126	
2: Exe	10	1	0	High	None	20	114	

Red font indicates active patient as of 8 October 13. High = >67%, medium = 33%–67%, low = <33%. [a]Patient switched to 50 mg exe; [b]previously treated with same AI; [c]HER2 amplified; [d]tested using automated quantitative analysis. aBC=advanced breast cancer; AI=aromatase inhibitor; ana=anastrozole; AR=androgen receptor; ER=estrogen receptor; exe=exemestane; HER2=human epidermal growth factor receptor 2; PgR=progesterone receptor.

Figure 7. Androgen receptor staining



Representative AR staining (imaged at 60×). 4 µm formalin-fixed paraffin-embedded sections were cut and stained with anti-AR clone AR441 (Dako) using Clarient CLIA AR immunohistochemistry assay. For determination of AR expression, only nuclear intensity and a proportion of positive staining in invasive tumor cells were evaluated. Samples ≥10% nuclear staining were scored as positive.

Distribution of samples based on nuclear staining. Patients were grouped according to their percentage of AR+ nuclear staining irrespective of their intensities. Patients whose tissue sample was from bone were excluded from this analysis.
ana=anastrozole; AR=androgen receptor; enza=enzalutamide; exe=exemestane.

CONCLUSIONS

- Enzalutamide, taken daily at a dose of 160 mg, is generally well tolerated as a single agent and in combination with either ana or exe.
  - No seizures have been observed to date.
- Enzalutamide can be combined with exe and effective aromatase inhibition is maintained.
  - Due to the observed decrease in exposure, the study has been expanded to evaluate 50 mg exe plus enzalutamide; data expected in 2014.
- Enzalutamide reduced mean exposure to ana by ≈88% and the effects on hormones were variable.
- AR expression in this aBC population is representative of that reported in the literature.[3,7]
- 12 of the 36 patients enrolled into the AI cohorts have been on study for 16 weeks or longer.
- Three global phase 2 clinical trials are enrolling:
  - Protocol MDV3100-11: single-agent enzalutamide in AR+ TNBC (primary endpoint: clinical benefit rate).
  - Protocol MDV3100-12: a randomized trial investigating exe plus enzalutamide versus exe plus placebo in hormone-receptor–positive breast cancer (primary endpoint: progression-free survival).
  - Protocol 9785-CL-112: an open-label study investigating enzalutamide with trastuzumab in HER2+ AR+ metastatic or locally advanced breast cancer (primary endpoint: clinical benefit rate ≥24 weeks).

Acknowledgments

Enzalutamide is co-developed by Medivation, Inc. and Astellas. This study was funded by Medivation, Inc. Editorial assistance funded by Medivation, Inc. and Astellas was provided by Shannon Davis of Infusion Communications.

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